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PHASE 3 SUMMARY OF MRID 00094009 AND RELATED MRIDS 00146630 AND 00153878:

AMES SALMONELLA/MICROSOME PLATE TEST

STUDY # 301-CG-001-81/A

FLUMETRALIN

GUIDELINE REFERENCE:

84-2(A) GENE MUTATION - AMES

SUMMARY PREPARED BY:

CHARLES BRECKENRIDGE, Ph.D.

DEMETRA VLAVHOS

5 OCTOBER 1990

ORIGINAL STUDY PREPARED BY:

PHARMAKON RESEARCH INTERNATIONAL, INC.

WAVERLY, PENNSYLVANIA

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: CIBA-GEIGY Corporation (Typed Name)

Company Agent: Thomas Parshley (Typed Name)

Title: Senior Reg. Specialist

Signature: _____ Date: _____

These data are the property of the Agricultural Division of CIBA-GEIGY Corporation, and as such, are considered to be confidential for all purposes other than compliance with FIFRA §10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE STATEMENT

This study meets the requirement for 40 CFR Part 160.

Submitter/Sponsor of Study:

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COMPLIANCE STATEMENT

This study was conducted in compliance with the Principles of Good Laboratory Practices (GLP) as promulgated by the following regulatory agencies:

U.S. Food and Drug Administration, as stated in the Federal Register, Part II of December 22, 1978, Title 21, part 58 and all subsequent revisions.

U.S. Environmental Protection Agency as stated in the Federal Register, Vol. 48, No. 230, Tuesday, November 29, 1983.

Organization for Economic Co-operation and Development Guidelines for Testing Chemicals (OECD), ISBN 92-64-12221-4, adopted by the council at its 535th meeting on 12th May, 1981.

Study No.: PH 301-CG-001-81
PH 301-CG-001-81A

"To the best of my knowledge, this study was conducted in accordance with applicable Good Laboratory Practice regulations; there were no deviations from these regulations that impacted on study conclusions."

Study Director

Date 9/6/90

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Certification of Availability of Raw Data

I hereby certify that the submitter possesses or has access to the raw data used in or generated by the study summarized in this document.

Submitter's Representative:

Signature/Date: Merrill Tisdel 10.15.90

Typed Name: Merrill Tisdel

Title: Toxicologist

Certification of Accuracy of Summary and Adequacy of the Study

I certify, in compliance with FIFRA section 4(e)(1)(A), that this summary accurately represents the data presented in the report(s) of this study cited by MRID, and that this study fully satisfies all pertinent requirements of the OPP Guideline it addresses.

Submitter's Representative:

Signature/Date: Merrill Tisdel 10.15.90

Typed Name: Merrill Tisdel

Title: Toxicologist

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Subdivision F
Guideline Ref. No. 84-2
December 24, 1989

84-2 Mutagenicity Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

General Requirements

1. Y Technical form of the active ingredient tested.
2. Y Negative, solvent and/or vehicle control(s) for the test system.
3. Y Positive control(s) for the test system.
4. Y Fully identified test system, species, strain, source etc.
5. Y Fully described method for maintaining test system.
6. Y Fully described method for preparing test environment and administering test compond.
7. Y Fully described metabolic acitvation system, if required.
8. Y Determination of maximum and range of concentrations/doses used under test conditions.
9.* Y Criteria for determination of a positive effect.

Test Specific Requirements

Salmonella reverse mutation assay

1. Y Minimum of four strains, TA98, TA100, TA1535 and TA1536. (alternatives need rationale)
2. Y Strain specific positive controls.
3. Y Highest concentration limited by toxicity, solubility or 5000 ug/plate.
4.* Y At least 5 different concentrations of test material at adequate intervals.
5.* Y A single positive response confirmed by testing over a narrow range of concentrations.
6.* Y/N At least three plates experimantal point.

Gene mutation in somatic cells in culture

1. ___ Highest concentration limited by toxicity (10-20% relative survival), solubility or 5000 ug/ml.
2.* ___ At least 4 different concentrations of test material to yield a concentration related toxic effect.
3. ___ Determination of the number of cell cultures used.

In vitro mammalian cytogenetics

1. ___ Highest concentration limited by toxicity (e.g. reduced mitotic activity; alteration of cell cycle; cytotoxicity), solubility or 5000 ug/ml.
2.* ___ Multiple concentrtions used to define the response.
3.* ___ At least two independent cultures for each experimental point.
4. ___ Determination of culture harvest time.

Criteria marked with a * are supplemental and may not be required for every study.

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IDENTIFICATION OF TEST MATERIAL

Chemical Name

CAS Name: N-(2-Chloro-6-fluorobenzyl)-N-ethyl-α,α,α,-trifluoro-2,6-dinitro-p-toluidine

or

2-Chloro-N-[2,6-dinitro-4-(trifluoromethyl)phenyl]-N-ethyl-6-fluorobenzenemethanamine

Common Name: Flumetralin

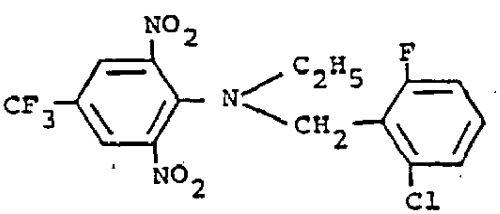
Trade Name: Prime +®

CIBA-GEIGY Code Number: CGA-41065

CAS Registry Number: 62924-70-3

EPA Shaughnessy Number: Unknown

Chemical Structure:



Percent Active Ingredient

92% minimum

Flumetralin: 84-2: Gene Mutation Test - Ames Assay

General Requirements

1. The test article was Flumetralin (CGA-41065) Technical, Batch FL-810824; it was 92.7% pure.

2. The vehicle for the test article was dimethoxysulfoxide (DMSO). DMSO was also used as the negative control with each test strain of Salmonella typhimurium evaluated.

3. The positive control substance used depended on the strain of bacteria and whether the metabolic activation mixture was added as indicated below:

Strain	Positive Control	Concentrations (µg/plate)
	NON-ACTIVATED SYSTEMS	
TA 100, TA 1535	N-Methyl-N'-nitro-N-nitrosoguanidine	2
TA 1537	9-aminoacridine	150
	METABOLICALLY ACTIVATED SYSTEM	
TA 98, TA 1538	2-aminofluorene	10

4. Five different strains of Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537 and TA 1538) were used. The original bacteria were obtained from Professor B. Ames, Berkeley, California, U.S.A.

5. The media used for each plate consisted of the following ingredients:

   a) minimal glucose plates.

   b) 2 ml top overlay agar supplemented with 0.5 mM histidine and 0.5 mM biotin in a volume of 0.1 ml/ml of agar.

c) 0.1 ml of the solution containing the test or the control article.

d) 0.1 ml of the appropriate bacterial culture.

e) In those experiments where the test material was metabolically activated, 0.5 ml of the S-9 activation mixture was added to the Petri dishes:

Each ml of the metabolic activation mixture consisted of the following ingredients:

(1) 40-100 µl of the liver S9 fraction (33 mg protein/ml) from Sprague-Dawley rats that had been induced with Aroclor 1254.

(2) 565 µl of a solution of cofactors.

(3) 335-395 µl of sterile distilled water.

Two plates per strain/concentration were prepared for the test groups and three plates were evaluated for the negative and positive control groups. The plates were incubated in the dark for 48 to 72 hours at 37°C. The number of revertants/plate was counted and the mean and standard deviation were calculated.

6. CGA-41065 formed a yellow precipitate when added to the aqueous top agar solution at concentrations of ≥333 µg/plate (3.33 mg/ml).

7. A positive response was indicated if there was a tripling over the negative control group of the mean number of revertants per plate at any test article concentration for any test strain. The response should be reproducible and generally display a concentration-dependent relationship.

Under the conditions of this experiment, CGA-41065 was not mutagenic at concentrations up to the solubility limit of 333 µg/plate. A slight positive response (less than three-fold increase) was noted at levels ≥10000 µg/plate for non-activated and activated test strains TA 98 and TA 1538.

Specific Requirements:

1. The strains of Salmonella typhimurium used in this study were TA 98, TA 100, TA 1535, TA 1537, and TA 1538.

2. Positive control substances tested are indicated in Item 3 under General Requirements.

3. CGA-41065 concentrations of 100, 333, 1000, 3333, and 10000 µg/plate were evaluated in each test strain, both with and without metabolic activation. In addition, concentrations of 100, 333, 1000, 3333, 10000, 12000, 14000, and 20000 µg/plate were evaluated in test strains TA 98 and TA 1538 in non-activated and activated media. Compound precipitation was noted at concentrations of ≥333 µg/plate. Although a slight increase in the number of revertants/plate occurred at concentrations of ≥10000 µg/plate for test strains TA 98 and TA 1538, using the current standard for study conduct (EPA Health Effects Test Guidelines, 40 CFR 798, 5265, 1987), CGA-41065 would be considered negative in this assay because of (a) the solubility limitation (<333 µg/plate), and (b) the 10000 µg/plate level exceeds the highest concentration required by guideline.

4. A single experiment was conducted for test strains TA 100, TA 1535, and TA 1537; a confirmatory experiment was performed with strains TA 98 and TA 1538 following the initial experiment. For each experiment, duplicate plates were evaluated for each test concentration of CGA-41065. Triplicate plates were evaluated for the negative (solvent) and positive controls.

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